Differential Effects of Naltrindole on Morphine-Induced Tolerance and Physical Dependence in Rats[1]

MATTHEW J. HEPBURN, PATRICK J. LITTLE, JEANINE GINGRAS and CYNTHIA M. KUHN

Departments of Pharmacology (M.J.H., P.J.L., C.M.K.) and Pediatrics (J.G.), Duke University Medical Center, Durham, North Carolina

Accepted for publication February 20, 1997

ABSTRACT

This study investigated the effect of *delta* opioid receptor blockade by naltrindole on the development of physical dependence and tolerance to the antinociceptive and respiratory depressive effects of morphine in rats. Chronic morphine was delivered either by s.c. injection of increasing amounts of morphine over 5 days or by s.c. implantation of morphine pellets. Animals were cotreated with saline or naltrindole. Antinociception and respiratory depression were assessed after administration of a challenge dose of morphine, and withdrawal signs were determined after naloxone challenge. Naltrindole significantly attenuated the development of antinociceptive tolerance after all three chronic treatment regimens. In addition, rats pretreated with naltrindole displayed significantly fewer with- drawal symptoms and less weight loss after a naloxone chal- lenge. In contrast, naltrindole did not prevent the development of tolerance to morphine-induced respiratory depression. These results imply that tolerance to antinociception and phys- ical dependence involves adaptations at interacting *mu* and *delta* receptor populations, whereas tolerance to respiratory depression reflects actions of independent *mu* and *delta* recep- tor populations. These findings suggest that *delta* antagonists may have potential clinical application for decreasing the rapid development of tolerance to opiate-induced analgesia, while allowing for the development of protective tolerance to respi- ratory depression.

There is increasing evidence of interaction between *mu* and *delta* opiate receptors (see Traynor and Elliot, 1993 for review). These receptors can coexist on the same neuron, as proposed for receptor populations in the neostriatum (Schof- felmeer *et al.*, 1990), or interact at different sites in a common pathway (Rossi *et al.*, 1994). Biochemical evidence also sup- ports the existence of independent and interacting *mu* and *delta* receptors. On the basis of radioligand binding studies, Rothman *et al.* (1988) suggest that *delta* opiate receptors may exist in two different states: complexed with *mu* receptor (d_{cx}) or independent of *mu* receptors (d_{ncx}).

Stimulation of *delta* opiate receptors modulates *mu*-based antinociception. Two populations of delta receptors ($delta_1$ and $delta_2$) have been postulated on the basis of pharmaco- logic evidence (Jiang *et al.*, 1991). Both *delta*-1 agonists, such as DPDPE, and *delta*-2 agonists, such as [D-Ala^2, Glu^4] del- torphin, have been demonstrated to interact with morphine (Jiang *et al.*, 1991; Porreca *et al.*, 1992), perhaps in a syner- gistic way (Malmberg and Yaksh, 1992). However 5'-NTII, an antagonist specific for the *delta*-2 receptor, blocked both ef-

fects (Porreca *et al.*, 1992). The agonist data suggest that *mu* and *delta* receptor populations can interact to produce an- tinociception. However, the failure of *delta* antagonists to block morphine-induced antinociception suggests that *delta* receptor function is not mandatory for *mu*-mediated antino- ciception (Calcagnetti and Holtzman, 1991; Jackson *et al.*, 1989; Jiang *et al.*, 1991).

There is also evidence that *delta* receptor activation con- tributes to the development of morphine-induced tolerance and physical dependence. Coadministration of the *delta*-2 antagonist 5'-NTII with either 100 mg/kg of morphine or morphine pellets over 3 days prevented the normal develop- ment of tolerance and dependence in mice, whereas the *delta*- 1-specific antagonist DALCE did not prevent the develop- ment of physical dependence in mice (Abdelhammid *et al.*, 1991; Miyamoto *et al.*, 1993, 1994). BW373U86, a putative *delta* opioid agonist, attenuated abstinence behaviors in rats when co-administered with morphine (Lee *et al.*, 1993). Sur- prisingly, administration of the *delta* antagonist naltrindole (Portoghese *et al.*, 1988) with morphine did not significantly block physical dependence in this same model system (Lee *et al.*, 1993). Although these findings support a role for *delta* receptors in opiate dependence, the effectiveness of a *delta* agonist in this regard appears to contradict the reported

ABBREVIATIONS: BW373U86, (±)-4-[(a-R*)-a-[(2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-hydroxybenzyl]-N,N-diethylbenzamide; DALCE, [D-Ala^2, Leu^5, Cys^6]enkephalin; [D-Ala^2, Glu^4] deltorphin, Tyr-D-Ala-Gly-Val-Val-Gly-NH$_2$; NTI, naltrindole hydrochloride; 5'-NTII, naltrindole-5'-isothiocyanate; d_{cx}, $d_{complexed}$; d_{ncx}, $d_{noncomplexed}$.

effects of *delta* antagonists. This discrepancy creates controversy as to the specific role of *delta* receptors in the development of tolerance.

Both endogenous and exogenous opioids are known to induce respiratory depression. One postulated mechanism is diminished sensitivity of the neurons of the brain stem to the stimulatory effects of carbon dioxide (for review see Shook *et al.*, 1990). However, the role of *delta* receptors in this effect is unclear. *Delta* agonists have been demonstrated to induce respiratory depression in rats whether they are administered centrally (Pazos and Florez, 1984) or peripherally (Morin-Surun *et al.*, 1984). The experiments of Ling *et al.* (1985) suggest that the *mu*-2 receptor is crucial in opiate-induced respiratory depression and that *delta* receptors are less important. However, there have been no studies investigating the role of *delta* receptor antagonists in the development of tolerance to opiate-induced respiratory depression.

The present study was conducted to investigate the effect of *delta* receptor blockade by naltrindole on the development of tolerance to morphine-induced antinociception, on respiratory depression and on the development of physical dependence during chronic morphine treatment of rats. The purposes of this study were to extend previous studies into rats and to investigate *delta* receptor effects on tolerance to respiratory depression as a system in which the acute effects of *delta* receptor function are known but chronic effects have not been documented. The results of this study suggest that naltrindole partially attenuates the development of tolerance to morphine-induced antinociception and physical dependence, while not affecting tolerance to morphine-induced respiratory depression.

Materials and Methods

Animals. Adult Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were used in all experiments. The rats had access to food and water *ad libitum* and were on a 12-hr light/dark cycle, with the lights on at 6:00 A.M.

Materials. Naltrindole hydrochloride was purchased from Research Biochemicals International (Natick, MA). Morphine and the morphine pellets were generously supplied by the National Institute on Drug Abuse. Morphine and naltrindole were dissolved in saline.

Chronic morphine injection paradigm. The chronic morphine regimen used was the paradigm shown previously to lead to significant tolerance and measurable withdrawal behaviors (Windh *et al.*, 1995). Morphine was administered s.c. at 12-hr intervals for 5 days. The dosage began at 5 mg/kg and was increased 5 mg/day up to 25 mg/kg on Day 5. The animals received no morphine on Day 6 and were challenged with morphine (10 mg/kg) on Day 7. Control animals were treated with saline. Subcutaneous naltrindole (1 mg/kg) or saline was given 1 hr before the morning morphine doses on Days 1 to 5. The naltrindole dose and daily treatment schedule are based on the long half-life of the drug and on effective blockade of the *delta* receptor by peripheral administration (Portoghese *et al.*, 1988).

Morphine pellet paradigm. One or two 75-mg pellets were implanted s.c. between the scapulas in animals anesthetized with isoflurane. Control animals received placebo pellets of identical size and weight.

Naltrindole administration. Naltrindole (10 µg) was administered *via* an i.c.v. route 90 min before pellet implantation. The i.c.v. naltrindole dose and regimen were based on experiments by Eisenberg (1993). For this procedure, animals were anesthetized with isoflurane and injected through the foramen magnum into the fourth ventricle. Control animals received i.c.v. saline. This procedure was

repeated 24 hr after the initial naltrindole administration. Animals received an acute morphine challenge of 10 mg/kg at 48 hr.

Antinociceptive assay. The warm-water tail-flick assay was used to measure antinociception (Janssen *et al.*, 1963; Negus *et al.*, 1993). Each rat was placed on a table with the tail hanging freely over the edge. Each rat was gently restrained as the tail was immersed in a beaker of water heated to 55°C. A foot pedal timer was activated upon immersion of the tail and stopped when the tail was withdrawn. During the chronic injection protocol, animals were tested 2 min before their acute morphine challenge and then 40 min after the acute challenge. A maximum withdrawal time of 15 sec was employed. Pelleted-implanted animals were tested at 4, 16, 24 and 48 hr after pelleting and 40 min after an acute challenge with morphine. Pilot studies suggested that tissue damage and subsequent hyperalgesia occurred in rats subjected to serial testing when latencies exceeded 7 sec. Because serial testing was employed for the pellet experiments, the cut-off time for all pellet studies was set at 7 sec.

Withdrawal. After the completion of antinociception testing, withdrawal was precipitated in animals that received two morphine or placebo pellets. Animals were placed in individual cages approximately 2 hr after the morphine treatment. They were allowed to acclimate to these cages for 10 min. Naloxone (5 mg/kg) s.c. was administered, and then the animals were observed for 10 min (Windh *et al.*, 1995). Each animal was observed for 20 sec of each minute. The following behaviors were recorded as present or absent at each minute for 10 min: ptosis, salivation and forepaw treading. Wet dog shakes were counted for total episodes in the 10-min period. At the end of 10 min, the rats were checked for additional withdrawal signs: diarrhea, sensitivity to touch and chromodacryorrhea. The rats were weighed before the morphine challenge and then 1 hr after the naloxone challenge.

Respiratory depression. Respiratory depression was measured 48 hr after pellet implantation and an acute challenge with morphine. A pressure-sensitive chamber was utilized to measure respiratory depression (Weese-Mayer *et al.*, 1992). The machine was initially calibrated for atmospheric pressure, animal temperature and animal weight. The chamber pressure deflection (proportional to tidal volume with appropriate calculation) was computer-sampled at 200 Hz in resting animals with no gross movements. Respiration was sampled for 15 sec in every 30-sec period. The rats were placed in the chamber and allowed to acclimate for 10 min. Respiration was measured over 5 min, and an average basal value was obtained. A 10-mg/kg morphine challenge was administered to the rat, and respiration was measured between 25 and 35 min after the injection, when pilot studies suggested that morphine effects on respiration were most evident. These values were averaged and compared to the basal average for that animal to calculate a percentage difference. Values are expressed as 100(minute ventilation after acute morphine challenge)/minute ventilation at base line.

Statistics. Two-way ANOVA was used to determine the effect of naltrindole *vs.* saline pretreatment on the development of tolerance to antinociception. Repeated-measures ANOVA was utilized for the antinociception experiments in which serial testing was employed. Scheffé's *post-hoc* test was performed when ANOVA indicated a significant interaction between groups. The Mann-Whitney U test was utilized for withdrawal data in which the response was quantitated. The χ-square test was applied to the withdrawal data that measured the presence or absence of a sign in the animal at 10 min after the naloxone challenge. The level of significance for all applicable statistical tests was set at $P < .05$. Paired t tests were used to compare respiratory rate before and after morphine challenge.

Results

Blockade of tolerance to morphine-induced antinociception by cotreatment with naltrindole. Naltrindole partially blocked the development of tolerance to the antino-

ciceptive effects of chronic morphine administration after all three morphine treatment paradigms. As shown in figure 1, rats given the morphine injection regimen showed a significantly smaller antinociceptive response to morphine than rats injected with saline. Although the antinociceptive response of rats treated daily with naltrindole alone was no different from control values, animals cotreated with morphine and naltrindole showed significantly greater antinociceptive responses (less tolerance) than animals treated with morphine alone.

Blockade of tolerance by naltrindole also was observed after the acute challenge in animals implanted with a single morphine pellet (fig. 2). Throughout the serial testing, the control groups (placebo pellet/saline i.c.v. and placebo pellet/ naltrindole i.c.v.) maintained a stable base-line nociceptive threshold with little fluctuation. In morphine-treated animals, a significant antinociceptive response was observed 6 hr after implantation, and latencies remained elevated relative to placebo-treated animals at 48 hr. Placebo-treated animals achieved a maximal response during the acute challenge, whereas the morphine-treated rats showed latencies only slightly above prechallenge values. Morphine/naltrindole-treated animals achieved an antinociceptive response significantly greater than animals treated with morphine alone.

The effect of naltrindole was even more apparent in animals implanted with two pellets (fig. 3). Animals receiving morphine alone showed a larger antinociceptive response 6 hr after implantation than animals that had received one pellet, but latencies approached control values by 48 hr. In contrast, animals cotreated with morphine and naltrindole maintained nearly maximal levels for 48 hr. Basal latencies did not change significantly. The acute challenge caused a maximal response in controls, a very slight response in animals receiving two morphine pellets and a response intermediate between these two levels in animals cotreated with morphine and naltrindole.
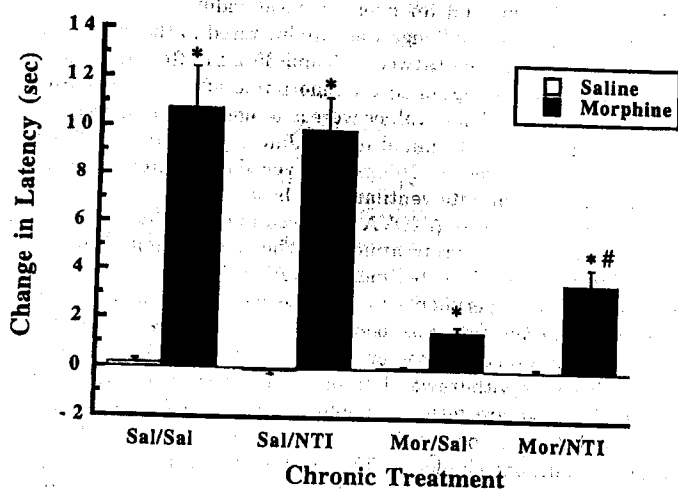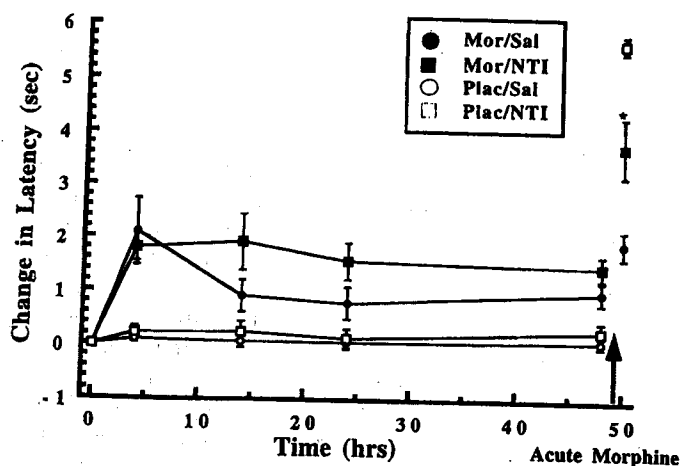
Fig. 2. Effects of naltrindole treatment on antinociceptive tolerance after implantation of a single morphine pellet. Antinociceptive response in rats implanted with one morphine or placebo pellet and cotreated with saline or naltrindole. Animals were treated with morphine and naltrindole as described in "Materials and Methods." Results are expressed as mean ± S.E.M. of the difference between pre- and post-treatment latency for individual animals. N = 6/experimental condition. * indicates P < .05 or better relative to morphine/saline.
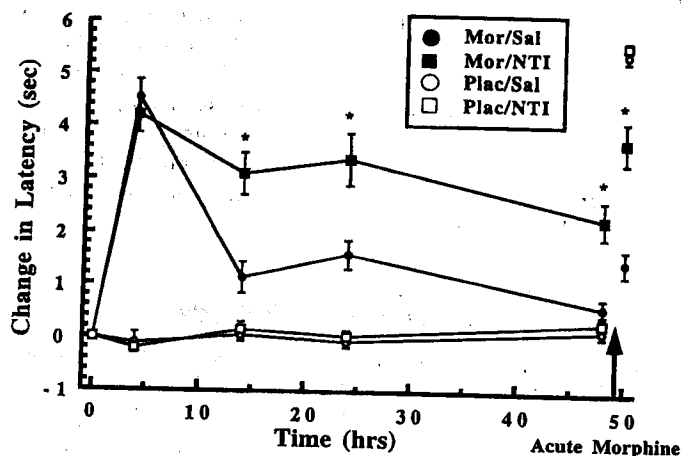
Fig. 3. Effects of naltrindole treatment on antinociceptive tolerance after two morphine pellets. Animals were treated with morphine and naltrindole as described in "Materials and Methods." Results are expressed as mean ± S.E.M. of the difference between pre- and post-treatment latency for individual animals. N = 8/experimental condition. * indicates P < .05 or better relative to morphine/saline.

Blockade of withdrawal by cotreatment with naltrindole. Cotreatment with naltrindole attenuated abstinence symptoms in the adult rats treated with the two-pellet paradigm. In animals exposed to morphine chronically, naloxone initiated significant withdrawal signs that were substantially more frequent than such signs in animals receiving placebo pellets (figs. 4 and 5). Seven measurements for withdrawal indicated a difference between the morphine/saline and morphine/naltrindole groups, including quantitated signs of forepaw treading, salivation, wet dog shakes (see fig. 4) and weight loss (see table 1), as well as the checked signs of diarrhea, vocalization on touch and chromodacryorrhea/ rhinorrhea (see fig. 5). These symptoms were present among the animals that received morphine and naltrindole, but the severity was diminished relative to the rats that received morphine alone. The degree of ptosis was not different between the two groups.
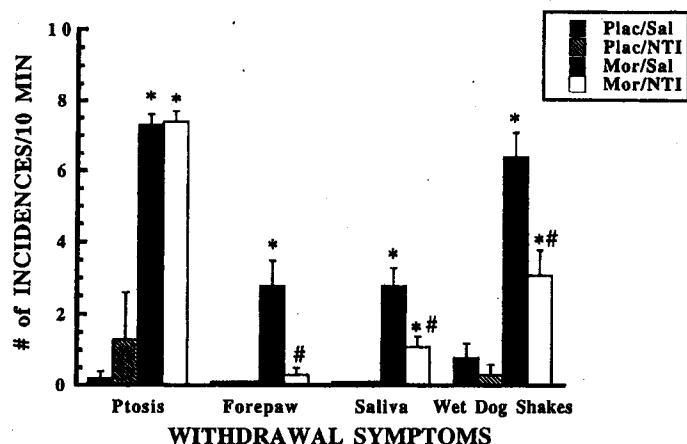
Fig. 1. Effects of naltrindole treatment on antinociceptive tolerance after the morphine injection paradigm. Antinociceptive response in rats treated with the increasing dose regimen of morphine for 5 days and cotreated with saline or naltrindole (s.c.) as described in "Materials and Methods." Results are expressed as mean ± S.E.M. of the difference between pre- and post-treatment latency for individual animals. N = 6/experimental condition. * indicates P < .05 or better relative to morphine/saline.

WITHDRAWAL SYMPTOMS

Fig. 4. Effects of naltrindole treatment on counted withdrawal signs. Animals were treated with two morphine or placebo pellets followed by naloxone as described in "Materials and Methods." Results are expressed as mean ± S.E.M. of incidences in 10 min. N = 10/experimental condition. * indicates P < .05 or better relative to matched placebo condition. # indicates P < .05 or better relative to morphine/saline treatment.
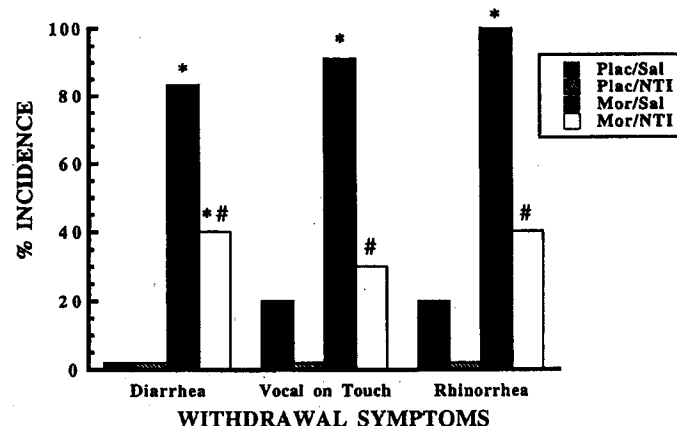

WITHDRAWAL SYMPTOMS

Fig. 5. Effects of naltrindole on checked withdrawal signs. Animals were treated with morphine or placebo pellets and with naltrindole or vehicle followed by precipitation of withdrawal as described in "Materials and Methods." Results are expressed as % incidence in the 10-min observation period. N = 10/experimental condition. * indicates P < .05 or better relative to matched placebo condition. # indicates P < .05 or better relative to morphine/saline treatment.

TABLE 1

Naltrindole effects on weight loss during withdrawal

Animals received two morphine or placebo pellets and naltrindole or saline. Change in weight (in grams) was recorded for 1 hr after naloxone precipitation. Results are expressed as mean ± S.E.M.

Chronic Treatment	Saline Cotreatment	Naltrindole Cotreatment
Placebo	7.2 ± 0.7	7.3 ± 1.5
Morphine	15.8 ± 1.5*	9.6 ± 0.8#

N = 10/experimental group.
* indicates P < .05 or better relative to placebo pellet controls.
# indicates P < .05 or better relative to morphine/saline.

Lack of naltrindole effects on tolerance to morphine-induced inhibition of respiration. The respiratory depression experiments were conducted on rats that were exposed to the two-pellet chronic morphine regimen (fig. 6). Base-line rates were obtained at 48 hr after the initial implantation. The similarities in the base-line values between the morphine-implanted animals and the placebo-implanted
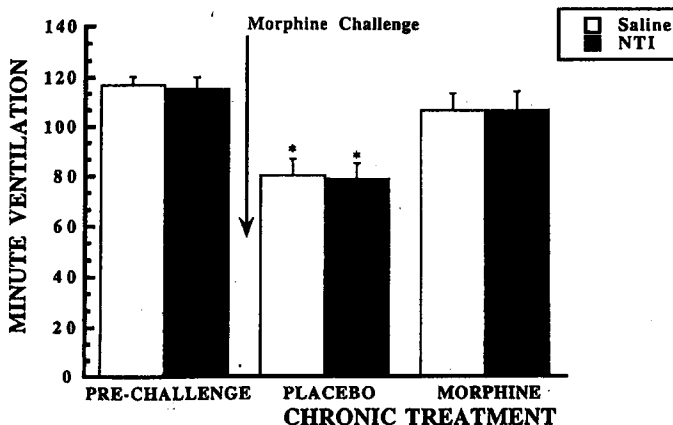

CHRONIC TREATMENT

Fig. 6. Effects of naltrindole on tolerance to respiratory depression. Animals were implanted with one morphine or placebo pellet and cotreated with naltrindole or vehicle as described in "Materials and Methods." Results are presented as mean ± S.E.M. of % change in respiratory rate for individual animals before and after a challenge dose of morphine. Basal respiratory rate = 117 ± 3 for placebo/saline, 106 ± 7 for morphine/saline, 115 ± 5 for placebo/naltrindole and 109 ± 5 for morphine/naltrindole. N = 5/experimental group. * indicates P < .05 or better relative to paired placebo condition.

animals suggests that rats develop rapid tolerance to the respiratory effects of morphine (see legend of fig. 6). The morphine-naive animals exhibited acute respiratory depression after the morphine challenge, whereas animals that received morphine pellets were not affected by the acute challenge.

Naltrindole itself did not affect respiration, and it had no effect on the acute respiratory depressive effects of morphine. Additionally, naltrindole did not inhibit the development of tolerance to respiratory depression in animals treated chronically with morphine. Morphine-pelleted animals did not experience respiratory depression after the acute challenge whether they received saline or naltrindole as a pretreatment.

Discussion

The findings of the present study suggest that occupation of *delta* opioid receptors contributes to the development of morphine-induced tolerance to antinociception and physical dependence in rats, whereas tolerance to morphine-induced respiratory depression is not influenced by *delta* receptor occupation. The findings of these experiments support the results of earlier studies in mice in which development of tolerance was prevented by *delta* receptor antagonists (Abdelhammid *et al.*, 1991). In addition, the present study extends previous findings of naltrindole blockade of naloxone-induced jumping to demonstrate blockade of additional *opioid* withdrawal signs. Finally, in contrast to the findings with antinociception, naltrindole cotreatment did not prevent the development of tolerance to morphine-induced respiratory depression.

The significant antinociception induced by morphine injection or pellet implantation was not influenced by coadministration of naltrindole. This is consistent with the majority of published studies, which indicate that although morphine-induced antinociception can be enhanced by administration of subantinociceptive doses of either of the *delta*-specific agonists DPDPE and [D-Ala^2, Glu^4] deltorphin (Heyman *et al.*,

1989; Porreca *et al.*, 1992; Malmberg and Yaksh 1992), administration of *delta* antagonists typically does not prevent morphine-mediated antinociception (Calcagnetti and Holtzmann, 1991; Abdelhammid *et al.*, 1991; Sofuoglu *et al.*, 1991).

The reason why *delta* receptor stimulation can augment *mu* agonist antinociception but *delta* antagonists do not diminish *mu*-mediated antinociception is not clear. Coupling of receptors has been demonstrated both at the level of the single cell and at the level of neural pathways. Rothman *et al.* (1988) have provided both *in vivo* and *in vitro* evidence of a coupling of *mu* and *delta* receptors. Similarly, Schoffelmeer *et al.* (1990, 1993) used biochemical techniques to demonstrate interacting *mu* and *delta* binding sites that inhibit dopamine-sensitive adenylate cyclase in the rat neostriatum. Alternatively, a *mu/delta* pathway interaction involving the periaqueductal gray and the rostral ventral medulla has been shown to augment morphine-induced antinociception (Rossi, 1994). However, the inability of *delta* antagonists to influence *mu*-mediated antinociception is not well understood.

Although naltrindole did not affect the acute antinociceptive response, it substantially decreased the development of tolerance after either peripheral injection of both morphine and naltrindole or i.c.v. administration of naltrindole before implantation of morphine pellets. These results provide support for the idea of *mu/delta* cooperativity in the development of morphine tolerance that has been previously demonstrated in mice (Abdelhammid *et al.*, 1991). These authors compared ED_{50} values for mice that had received morphine alone *vs.* those that received morphine and naltrindole or morphine and 5'NTII. They concluded that antagonism of *delta* receptors substantially prevented the development of tolerance.

The greater effectiveness of naltrindole administered after higher chronic morphine dose regimens in the present study was surprising. Although the blockade of tolerance after injection paradigms was quite modest, substantial blockade was observed after a single morphine pellet, and even better blockade occurred after the administration of two pellets. One reason might have been the different route used for naltrindole delivery, which probably delivered a significantly higher dose than that obtained after peripheral administration. However, the different blockade observed after one or two pellets is not easily explained pharmacokinetically. Whatever the mechanism, this quality offers potential clinical utility for improvement in analgesia in situations where increasing opiate doses are needed to deal with increasing pain.

Physical dependence is another aspect of chronic morphine administration that is susceptible to modification by *delta* receptor agonists or antagonists. In the present study, we found that naltrindole pretreatment significantly attenuated seven different withdrawal symptoms. These findings confirm and extend the report of Abdelhammid *et al.* (1991) that an increase in the amount of naloxone was needed to precipitate withdrawal jumping in morphine-dependent mice that were pretreated with naltrindole. This effect has been attributed to actions of the *delta*-2 receptor (Miyamoto *et al.*, 1993; Miyamoto *et al.*, 1994). It should be noted that contrasting conclusions have been drawn on the basis of studies with the putative *delta* agonist BW373U86. Lee *et al.* (1993) demonstrated that BW373U86 blocked the development of physical dependence, wherein coinfusion of naltrindole with morphine without BW373U86 did not prevent the development of tolerance to physical dependence in rats. However, the blockade of physical dependence by BW373U58 may be due to competitive antagonism at the *delta* receptor site, because this compound has been demonstrated to be a partial *mu* and *delta* agonist (Wild *et al.*, 1993).

The activity of naltrindole against a profile of withdrawal behaviors extends the previous findings with naloxone-induced jumping and suggests that interacting *mu*/delta receptors that are important for the development of opiate dependence exist at multiple sites in the CNS. Sites responsible for various opiate withdrawal behaviors are located both centrally and peripherally (Koob *et al.*, 1992). The withdrawal signs of diarrhea (Bianchetti *et al.*, 1986), salivation, lacrimation and rhinorrhea may be mediated by peripheral receptors (Maldonado *et al.*, 1992), whereas the locus ceruleus and the periaqueductal gray matter have also been implicated as sites that are active during opiate withdrawal (Maldonado *et al.*, 1992). The anterior preoptic and raphe magnus may be particularly important for the induction of wet dog shakes (Maldonado *et al.*, 1992). Our results suggest that cooperative *mu* and *delta* receptors may mediate withdrawal at several of these sites. However, the absence of actions on ptosis was a little surprising, because symptoms such as diarrhea that are thought to have a similar mediation by noradrenergic hyperactivity were blocked (Taylor *et al.*, 1988).

The conclusions of the present study regarding the role of *delta* opioid receptors rely in part on the reported specificities of morphine and naltrindole for *mu* and *delta* receptors, respectively. Morphine is reported to show 10-fold specificity for *mu* over *delta* receptors *in vitro* (Pasternak 1986, Change *et al.*, 1979), whereas naltrindole specificity ranges from 20-fold to 100- to 500-fold in different reports (Portoghese *et al.*, 1988; Ayers *et al.*, 1990; Rogers *et al.*, 1990). *In vivo*, a number of studies have shown that the present dose regimen for naltrindole fails to block analgesia by *mu* agonists while effectively blocking that induced by *delta* agonists (Portoghese *et al.*, 1988; Calcagnetti and Holtzmann, 1991; Drower *et al.*, 1991; Improta and Broccardo, 1992; Malmberg and Yaksh, 1992; Craft *et al.*, 1995; Yaksh *et al.*, 1995). The most relevant report for the present study is the recent demonstration that naltrindole and the more selective *delta* antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH) similarly block the development of morphine dependence during antagonist and morphine coinfusion into rats (Fundytus *et al.*, 1995). In this study, TIPP but not naltrindole prevented the development of tolerance to opiate-induced analgesia, but differences in the analgesic test conducted might explain this difference. Several factors in the present study support the validity of these assumptions. First, naltrindole did not influence morphine analgesia after the first 24 hr after morphine pellet implantation, when morphine brain levels are higher (Yoburn *et al.*, 1985). Therefore, morphine seems to have retained *mu* selectivity up to the highest levels observed in the present study. Similarly, naltrindole did not prevent morphine analgesia after an acute challenge of 10 mg/kg. The finding that a similar dose of naltrindole failed to block either morphine- or DAMGO-induced ACTH secretion supports the specificity of this *in vivo* naltrindole dose (C.M. Kuhn and R. Francis, unpublished observations). Nevertheless, a complete dose-response study for naltrindole-induced blockade of tolerance and the investigation of the ability of other *delta*

antagonists to prevent the development of tolerance would provide additional evidence bearing on this hypothesis.

Our experiments have demonstrated a significant tolerance to the morphine-induced respiratory depression in rats treated with two 75-mg morphine pellets—a tolerance that that was not prevented by coadministration of naltrindole. The tolerance observed resembles that previously reported in mice (Roerig *et al.*, 1987). The lack of naltrindole blockade suggests that the sites at which opiate agonists suppress respiration in the pons and medulla (Taviera da Silva *et al.*, 1983; Hurle *et al.*, 1982, 1985) may not possess interacting *mu* and *delta* receptor populations, although both *mu* and *delta* agonists are known to produce respiratory depression in rodents (Morin-Surun *et al.*, 1984; Pazos *et al.*, 1984). This conclusion is consistent with the putative receptor mediation of respiratory *vs.* antinociceptive effects, because agonists specific for the *delta*-1 receptor have been implicated in control of respiration (Mayfield and D'Alecy, 1994a; Mayfield and D'Alecy, 1994b), whereas *delta*-2 receptors have been implicated in tolerance and dependence (Miyamoto *et al.*, 1993; 1994). These results imply that independent *mu* and *delta* receptors regulate the development of tolerance to morphine-induced respiratory depression.

The finding of blockade of tolerance to antinociception but not respiratory depression has potential clinical significance, regardless of the relative role of *mu* and *delta* receptors in mediating this effect. Our findings suggest that coadministration of effective *mu* agonists in conjunction with *delta* antagonists could enhance the effectiveness of long-term therapy in which development of tolerance can impair the clinical effectiveness of drugs. The failure to block tolerance to respiratory depression would permit beneficial tolerance to a limiting side effect to develop, while maintaining clinical effectiveness. However, it should be emphasized that more studies using models of chronic pain would be required to demonstrate the utility of this treatment. The effectiveness of antagonists in blocking responses to an acute noxious stimulus are not necessarily predictive of responses in chronic pain models.

In summary, the present findings have demonstrated that development of tolerance to certain effects during chronic morphine administration relies on *mu/delta* cooperativity, whereas tolerance to other effects develops independently of *delta* receptors. This difference may create a window of opportunity for drug development. A *delta* antagonist may be useful in preventing the development of tolerance to morphine-induced analgesia, while minimizing withdrawal symptoms and avoiding the deleterious consequences of respiratory depression.

References

ABDELHAMMID, E. E., SULTANA, M., PORTOGHESE, P. S. AND TAKEMORI, A. E.: Selective blockade of *delta* opioid receptors prevents the development of morphine tolerance and dependence in mice. J. Pharmacol. Exp. Ther. **258**: 299–303, 1991.

AYERS, E. A., DAVIS, P. AND BURKS, T. F.: *In vivo* and *in vitro* investigation of naltrindole, a δ-opioid antagonist. Proc. West. Pharmacol. Soc. **33**: 55–63, 1990.

BIANCHETTI, A., GIUDICE, A., NAVA, F. AND MANARA, L.: Dissociation of morphine withdrawal diarrhea and jumping in mice by the peripherally selective opioid antagonist, SR 58002 C. Life Sci. **39**: 2297–2303, 1986.

CALCAGNETTI, D. J. AND HOLTZMAN, S. G.: *Delta* opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine. Pharmacol. Biochem. Behav. **38**: 185–190, 1991.

CHANG, K. J., COOPER, B. R., HAZUM, E. AND CUATRECASAS, P.: Multiple opiate receptors: Different regional distribution in the brain and differential binding of opiates and opioid peptides. Mol. Pharmacol. **16**: 91–104, 1979.

CRAFT, R. M., HENLEY, S. R., HAASETH, R. C., HRUBY, V. J. AND PORRECAM, F.: Opioid antinociception in a rat model of visceral pain: Systemic versus local drug administration. J. Pharmacol. Exp. Ther. **275**: 1535–1542, 1995.

DROWER, E. J., STAPEFELKO, A., RAFFERTY, M. F., DECOSTA, B., RICE, K. C. AND HAMMOND, D. L.: Selective antagonism by naltrindole of the antinociceptive effects of the delta opioid agonist cyclic [D-Penicillamine²-D-Penicillamine⁵] enkephalin in the rat. J. Pharmacol. Exp. Ther. **259**: 725–731, 1991.

EISENBERG, R. M.: DAMGO stimulates the hypothalamo-pituitary-adrenal axis through a *mu*-2 opioid receptor. J. Pharmacol. Exp. Ther. **266**: 985–991, 1993.

FUNDYTUS, M. E., SCHILLER, P. W., SHAPIRO, M., WELTROWSKA, G. AND CODERRE, T. J.: Attenuation of morphine tolerance and dependence with the highly selective δ-opioid receptor antagonist TIPP [Ψ]. Eur. J. Pharmacol. **286**: 105–108, 1995.

HEYMAN, J. S., VAUGHT, J. L., MOSBERG, H. I., RAAESTH, R. C. AND PORRECA, F.: Modulation of μ-mediated antinociception by δ agonists in the mouse: Selective potentiation of morphine and normorphine by [D-Pen², D-Pen⁵] enkephalin. Eur. J. Pharmacol. **165**: 1–10, 1989.

HURLE, M. A., MEDIAVILLA, A. AND FLORES, J.: Morphine, pentobarbital and naloxone in the ventral medullary chemosensitive areas: Differential respiratory and cardiovascular effects. J. Pharmacol. Exp. Ther. **220**: 642–647, 1982.

HURLE, M. A., MEDIAVILLA, A. AND FLORES, J.: Differential respiratory patterns induced by opioids applied to the ventral medullary and dorsal pontine surfaces of cats. Neuropharmacology **24**: 597–606, 1985.

IMPROTA, G. AND BROCCARDO, M.: Spinal antinociceptive effects of [D-Ala²] deltorphinII, a novel and highly selective *delta*-opioid receptor agonist. Peptides **13**: 1123–1126, 1992.

JACKSON, H. C., RIPLEY, T. L. AND NUTT, D. J.: Exploring *delta* receptor function using the selective opioid antagonist naltrindole. Neuropharmacology **28**: 1427–1430, 1989.

JANSSEN, P. A., NIEMEGEERS, C. J. AND DONY, J. G.: The inhibitory effect of fentanyl and other morphine-like analgesics on the warm-water induced tail withdrawal reflex in rats. Azneim. Forsch. **13**: 502–507, 1963.

JIANG, Q., TAKEMORI, A. E., SULTANA, M., PORTOGHESE, P. S., BOWEN, W. D., MOSBERG, H. I. AND PORRECA, F.: Differential antagonism of opiate *delta* antinociception by [D-Ala², Leu⁵, Cys⁶] enkephalin and naltrindole 5'-isothiocyanate: Evidence for *delta* receptor subtypes. J. Pharmacol. Exp. Ther. **257**: 1069–1075, 1991.

KOOB, G. F., MALDONADO, R. AND STINUS, L.: Neural substrates of opiate withdrawal. TINS **15**: 186–191, 1992.

LEE, P. H. K., McNUTT, R. W. AND CHANG, K.: A nonpeptidic *delta* opioid receptor agonist, BW373U86, attenuates the development and expression of morphine abstinence precipitated by naloxone in rat. J. Pharmacol. Exp. Ther. **267**: 883–887, 1993.

LING, G. S. F., SPIEGEL, K., LOCKHART, S. H. AND PASTERNAK, G. W.: Separation of opioid analgesia from respiratory depression: Evidence for different receptor mechanisms. J. Pharmacol. Exp. Ther. **232**: 149–155, 1985.

MALDONADO, R., STINUS, L. AND KOOB, G. F.: Role of different brain structures in the expression of the physical morphine withdrawal syndrome. J. Pharmacol. Exp. Ther. **261**: 669–677, 1992.

MALMBERG, A. B. AND YAKSH, T. L.: Isobolographic and dose-response analyses of the interaction between intrathecal *mu* and *delta* agonists: Effects of naltrindole and its benzofuran analog (NTB). J. Pharmacol. Exp. Ther. **263**: 264–275, 1992.

MAYFIELD, K. P. AND D'ALECY, L. G.: *Delta*-1 opioid receptor dependence of acute hypoxic adaptations. J. Pharmacol. Exp. Ther. **268**: 74–77, 1994a.

MAYFIELD, K. P. AND D'ALECY, L. G.: *Delta*-1 opioid agonist acutely increases hypoxic tolerance. J. Pharmacol. Exp. Ther. **268**: 683–688, 1994b.

MIYAMOTO, Y., BOWEN, W. D., PORTOGHESE, P. S. AND TAKEMORI, A. E.: Lack of involvement of *delta*-1 opioid receptors in the development of physical dependence on morphine in mice. J. Pharmacol. Exp. Ther. **270**: 37–39, 1994.

MIYAMOTO, Y., PORTOGHESE, P. S. AND TAKEMORI, A. E.: Involvement of *delta*-2 opioid receptors in acute dependence on morphine in mice. J. Pharmacol. Exp. Ther. **265**: 1325–1327, 1993.

MORIN-SURUN, M. P., BOUDINOT, E., GACEL, G., CHAMPAGNAT, J., ROQUES, B. P. AND DENAVIT-SAUBIE, B.: Different effects of *mu* and *delta* opiate agonists on respiration. Eur. J. Pharmacol. **98**: 235–240, 1984.

NEGUS, S. S., PASTERNAK, G. W., KOOB, G. F. AND WEINGER, M. B.: Antagonist effects of β-funaltrexamine and naloxonazine on alfentanil-induced antinociception and muscle rigidity in the rat. J. Pharmacol. Exp. Ther. **264**: 739–745, 1993.

PAZOS, A. AND FLOREZ, J.: A comparative study in rats of the respiratory depression and analgesia induced by *mu* and *delta* agonists. Eur. J. Pharmacol. **99**: 15–21, 1984.

PASTERNAK, G. W.: Multiple *mu* opiate receptors: Biochemical and pharmacological evidence for multiplicity. Biochem. Pharmacol. **35**: 361–364, 1986.

PORRECA, F., TAKEMORI, A. E., SULTANA, M., PORTOGHESE, P. S., BOWEN, W. D. AND MOSBERG, H. I.: Modulation of *mu*-mediated antinociception in the mouse involves opioid *delta*-2 receptors. J. Pharmacol. Exp. Ther. **263**: 147–152, 1992.

PORTOGHESE, P. S., SULTANA, M. AND TAKEMORI, A. E.: Naltrindole, a highly

selective and potent non-peptide *delta* opioid receptor antagonist. Eur. J. Pharmacol. **146:** 185–186, 1988.

ROERIG, S. C., FUJIMOTO, J. M. AND LANGE, D. G.: Development of tolerance to respiratory depression in morphine- and etorphine-pellet-implanted mice. Brain Res. **400:** 278–284, 1987.

ROGERS, H., HAYES, A. G., BIRCH, P. J., TRAYNOR, J. R. AND LAWRENCE, A. J.: The selectivity of the opioid antagonist naltrindole for δ-opioid receptors. J. Pharm. Pharmacol. **42:** 358–359, 1990.

ROSSI, G. C., PASTERNAK, G. W. AND BODNAR, R. J.: *Mu* and *delta* opioid synergy between the periaqueductal gray and the rostro-ventral medulla. Brain Res. **665:** 85–93, 1994.

ROTHMAN, R. B., LONG, J. B., BYKOV, V., JACOBSON, A. E., RICE, K. C. AND HOLIDAY, J. W.: β-FNA binds irreversibly to the opiate receptor complex: *In vivo* and *in vitro* evidence. J. Pharmacol. Exp. Ther. **247:** 405–416, 1988.

SCHOFFELMEER, A. N., DEVRIES, T. J., HOGENBOMM, F. AND MULDER, A. H.: *Mu* and *delta*-opioid receptors inhibitorily linked to dopamine-sensitive adenylate cyclase in rat striatum display a sensitivity profile toward endogenous opioid peptides different from that of presynaptic *mu, delta* and *kappa* receptors. J. Pharmacol. Exp. Ther. **267:** 205–210, 1993.

SCHOFFELMEER, A. N., YAO, Y.-H., GIOANNINI, T. L., HILLER, J. M., OFRI, D., ROQUES, B. P. AND SIMON, E. J.: Cross-linking of human [^{125}I] b-endorphin to opioid receptors in rat striatal membranes: Biochemical evidence for the existence of a *mu/delta* opioid receptor complex. J. Pharmacol. Exp. Ther. **253:** 419–426, 1990.

SHOOK, J. E., WATKINS, W. D. AND CAMPORESI, E. M.: Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. Am. Rev. Respir. Dis. **142:** 895–909, 1990.

SOFUOGLU, M., PORTOGHESE, P. S. AND TAKEMORI, A. E.: Differential antagonism of *delta* opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: Evidence for *delta* opioid receptor subtypes. J. Pharmacol. Exp. Ther. **257:** 676–680, 1991.

TAYLOR, J. R., ELSWORT, J. D., GARCIA, E. J., GRANT, S. J., ROTH, R. H. AND REDMOND, D. E.: Clonidine infusions into the locus coeruleus attenuate behavioral and neurochemical changes associated with naloxone-precipitated withdrawal. Psychopharmacology **96:** 121–134, 1988.

TAVIERA DA SILVA, A. M., SOUZA, J. D., QUEST, J. A., PAGANI, F. D., MOERSCHBAECHER, J. M., BULLER, A., HAMOSH, P. AND GILLIS, R. A.: Central nervous site for action for the respiratory depressant effect of diacetylmorphine (heroin) in the cat. J. Clin. Invest. **72:** 1209–1217, 1983.

TRAYNOR, J. R. AND ELLIOT, J.: *Delta*-opioid receptor subtypes and cross-talk with *mu*-receptors. TIPS **14:** 84–85, 1993.

WEESE-MAYER, D. E., KLEMKA-WALDEN, L. M., BARKOV, G. A. AND GINGRAS, J. L.: Effects of prenatal cocaine on the ventilatory response to hypoxia in newborn rats. Dev. Pharmacol. Ther. **18:** 116–124, 1992.

WILD, K. D., McCORMICK, J., BILSKY, E. J., VANDERAH, T., McNUTT, R. W., CHANG, K.-J. AND PORRECA, F.: Antinociceptive actions of BW373U86 in the mouse. J. Pharmacol. Exp. Ther. **267:** 858–863, 1993.

WINDH, R. T., LITTLE, P. J. AND KUHN, C. M.: The ontogeny of *mu* opiate tolerance and dependence in the rat: Antinociceptive and biochemical studies. J. Pharmacol. Exp. Ther. **273:** 1361–1374, 1995.

YAKSH, T. L., MALMBERG, A. B., RO, S., SCHILLER, P. AND GOODMAN, M.: Characterization of the spinal antinociceptive activity of constrained peptidomimetic opioids. J. Pharmacol. Ther. Exp. **275:** 63–72, 1995.

YOBURN, B. C., CHEN, J., HUANG, T. AND INTURRISI, C. E.: Pharmacokinetics and pharmacodynamics of subcutaneous morphine pellets in the rat. J. Pharmacol. Exp. Ther. **235:** 282–286, 1985.